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Western Blot (WB) Protocol

A straightforward WB protocol from sample denaturation to blotting.

A western blot (WB) lets you detect and evaluate the size of specific proteins in cell or tissue extracts. An important and widely used tool in biology, western blotting involves separating proteins according to their size via gel electrophoresis and then transferring them to a membrane to detect with specific antibodies.

Sample Denaturation

1. Heat the samples in Laemmli buffer at 70–100°C for 10 minutes.
2. Load the samples into the gel*.

   **Load 80–100 µg tissue lysate/lane or lysate from 2–5 x 10^5 cells/lane*

SDS-PAGE

1. Run the gel according to the manufacturer's instructions.

Transfer

1. Transfer to a nitrocellulose membrane at 200 mA for 2.5 hours at 4°C for wet transfer.
   For dry transfer, follow the manufacturer's instructions.

   Note: High molecular weight proteins may need a longer transfer time (see Western Blot Protocol for High Molecular Weight (HMW) Proteins)

Western blotting

1. Block the membrane with Blocking Solution (PBS with 3% BSA and 0.05% NaN_3) for 2–5 hours at room temperature with gentle agitation.
2. Add the primary antibody diluted in PBS 1% BSA, 0.1% Tween-20, and 0.05% NaN_3.

   Note: If you use a blocking peptide as a negative control, refer to our Peptide Blocking Protocol for WB.
3. Incubate for 2–3 hours at room temperature or overnight at 4°C with gentle agitation.
4. Wash the membrane with Washing Buffer (PBS and 0.1% Tween-20) for 3 x 10 minutes at room temperature.

   Note: If you are using a secondary antibody conjugated to HRP, do not use solutions containing NaN_3 from this point on.
5. Incubate the secondary antibody in PBS with 1% BSA and 0.1% Tween-20 for 1 hour at room temperature with gentle agitation.
6. Wash the membrane with Washing Buffer for 3 x 10 minutes at room temperature.
7. Proceed to detection using an enhanced chemiluminescence (ECL) system.

   Note: If using a commercial kit, follow the manufacturer's instructions.
8. The membrane exposure time to the film/imager will depend on the abundance of the protein and the detection system.

Example data

Figure 1
WB analysis of mouse brain membranes (lanes 1 and 3) and rat brain membranes (lanes 2 and 4).

1-2. Anti-OCTN1/SLC22A4 Antibody (ACT-014), (1:600).

3-4. Anti-OCTN1/SLC22A4 Antibody, preincubated with OCTN1/SLC22A4 Antibody Blocking Peptide (BLP-CT014).